Journal of Medical Virology 59:527–535 (1999)

Genetic Investigation of Novel Hantaviruses Causing Fatal HPS in Brazil

Angela M. Johnson,[1] Luiza T. M. de Souza,[2] Ivani B. Ferreira,[2] Luiz E. Pereira,[2] Thomas G. Ksiazek,[1] Pierre E. Rollin,[1] C. J. Peters,[1] and Stuart T. Nichol[1]*

[1]*Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia*
[2]*Servico de Virologia, Instituto Adolfo Lutz, São Paulo, Brazil*

Although hantavirus pulmonary syndrome (HPS) was discovered in North America in 1993, more recent investigations have shown that the disease is a much larger problem in South America, where a greater number of cases and HPS-associated viruses have now been detected. Here we describe the genetic investigation of three fatal HPS cases from Brazil, including a 1995 case in Castelo dos Sonhos (CAS) in the state of Mato Grosso and two 1996 cases in the counties of Araraquara (ARA) and Franca (FRA), in the state of São Paulo. Reverse transcription-polymerase chain reaction (RT-PCR) products representing fragments of the hantavirus N, G1, and G2 coding regions were amplified from patient acute-phase serum samples, and the nucleotide (nt) sequences (394, 259, and 139 nt, respectively) revealed high deduced amino acid sequence identity between ARA and FRA viruses (99.2%, 96.5%, and 100%, respectively). However, amino acid differences of up to 14.0% were observed when ARA and FRA virus sequences were compared with those of the geographically more distant CAS virus. Analysis of a 643-nt N coding region and a 1734-nt predominantly G2-encoding region of ARA and CAS virus genomes confirmed that these Brazilian viruses were distinct and monophyletic with previously characterized Argentinean hantaviruses, and suggested that Laguna Negra (LN) virus from Paraguay was ancestral to both the Brazilian and Argentinean viruses. The phylogenetic tree based on the N coding fragment also placed LN in a separate clade with Rio Mamore virus from Bolivia. At the amino acid level, ARA and CAS viruses appeared more closely related to the Argentinean viruses than they were to each other. Similarly, analysis of the diagnostic 139-nt G2 fragment showed that the Juquitiba virus detected in a 1993 fatal HPS case close to São Paulo city, Brazil was closer to Argentinean viruses than to ARA or CAS viruses. These data indicate that at least three different hantavirus genetic lineages are associated with Brazilian HPS cases. ***J. Med. Virol. 59:527–535, 1999.***

KEY WORDS: hantavirus pulmonary syndrome; PCR; phylogenetic analysis

INTRODUCTION

Hantaviruses, family *Bunyaviridae*, have become recognized as a worldwide disease problem. For many years, hantaviruses have been implicated as the cause of hemorrhagic fever with renal syndrome (HFRS) in the Old World. Hantaan (HTN), Seoul (SEO), Dobrava (DOB), and Puumala (PUU) viruses are well known for their association with HFRS of varying degrees of severity (reviewed in Schmaljohn and Hjelle [1997]). The reservoirs of these viruses are rodents belonging to the *Murinae* (HTN, SEO, DOB) and *Arvicolinae* (PUU) subfamilies of the family *Muridae* (reviewed in Hjelle et al. [1995b], Plyusnin et al. [1996], and Schmaljohn and Hjelle [1997]).

Hantaviruses are now recognized in the Americas as causing a respiratory disease known as hantavirus pulmonary syndrome (HPS). Since 1993, when HPS was first identified in the New World [Nichol et al., 1993], many new hantaviruses have been described throughout North, Central, and South America. The list of HPS-associated viruses include Sin Nombre (SN), Black Creek Canal (BCC), Bayou (BAY), and New York (NY) viruses in North America and Laguna Negra (LN), Andes (AND), Lechiguanas (LEC), and Oran (ORN) in South America [Nichol et al., 1993; Hjelle et al., 1995d; Morzunov et al., 1995; Ravkov et al., 1995; Johnson et al., 1997; López et al., 1996; Levis et al.,

*Correspondence to: Stuart T. Nichol, Special Pathogens Branch, MS G-14, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30329-4018. E-mail stn1@cdc.gov

Accepted 13 April 1999

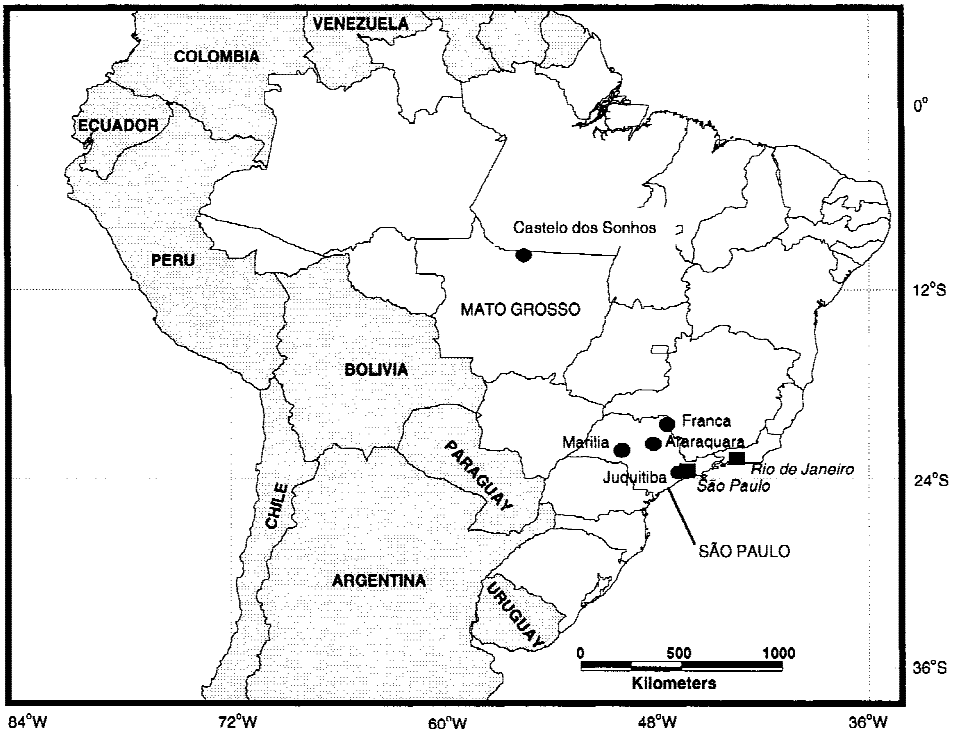

Fig. 1. Location of hantavirus pulmonary syndrome (HPS) cases. Black ovals indicate location of case residence or probable site of exposure. Black squares indicate the major cities of Rio de Janeiro and São Paulo. The states of Mato Grosso and São Paulo are also indicated.

1998; Monroe et al., 1999]. All of these New World hantaviruses have rodents of the subfamily *Sigmodontinae* as their natural hosts. Several additional hantaviruses have been detected in rodents in the Americas but have yet to be associated with human disease. The genome of these viruses consist of three single-stranded RNA segments, S, M, and L, which encode the nucleoprotein (N), glycoproteins (G1 and G2), and the polymerase protein, respectively [Elliott et al., 1991].

HPS was first discovered in South America with the confirmation of a fatal HPS case in São Paulo state, Brazil, in 1993 [Nichol et al., 1996; da Silva et al., 1997; Vasconcelos et al., 1997]. Genetic characterization of the virus detected in this case (referred to as Juquitiba [JUQ]), was limited to a 139-nt piece of the G2 encoding region of the virus [Monroe et al., 1999]. The focus of the current study was to carry out a genetic investigation of viruses associated with three more recent fatal HPS cases in Brazil, and to gain insight into the genetic diversity of HPS-associated hantaviruses in Brazil and elsewhere in South America.

MATERIALS AND METHODS

Human Specimens, RNA Extraction, and Genetic Analysis

Three serum specimens were tested by enzyme-linked immunosorbent assay (ELISA) for antibodies cross-reactive with SN virus N protein [Johnson et al., 1997]. The first (9618005) was from a suspected HPS case-patient that occurred in 1995; the patient resided in the settlement of Castelo dos Sonhos (CAS) in the state of Mato Grosso (Fig. 1). The other two samples were from cases that occurred further southeast in the state of São Paulo in 1996. One of these (9618006) was from a woman who resided in Araraquara (ARA). The other sample (9618007) was from a veterinary student who spent time both in Marilia, where he was attending classes, and in Franca (FRA) at his family's farm. Activities at both locations involved dealing with horses and cows in rural settings [Katz et al., unpublished data]. As the exact site of the patient's virus exposure is unknown, the case is referred to as FRA, based on detection of hantavirus-specific antibody in 3 of 55 contacts tested at this location, and the presence of subfamily *Sigmodontinae* rodents at this residence.

After HPS confirmation by serological testing, RNA was extracted from the serum samples as described previously [Johnson et al., 1997]. Reverse transcription (RT), first- and second-round polymerase chain reaction (PCR), and product purification were also performed as described previously [Johnson et al., 1997]. A QIAEX II gel extraction kit (QIAGEN Inc., Chatsworth, Calif.) was also used for some of the PCR product purifications. Samples were tested with primers designed to detect hantaviruses associated with sigmodontine rodents [Johnson et al., 1997]. Primers designed to detect part of the G2 portion of the M segment of BAY virus were also used [Morzunov et al., 1995; Fulhorst et al., 1997]. To extend the sequence information, more specific primers were designed based on nucleotide sequences determined from the diagnostic PCR fragments generated. Direct sequencing of the pu-

rified PCR products was done on an ABI 377 sequencer by using the dye termination cycle sequencing technique (Applied Biosystems, Inc., Foster City, CA). Genetic sequences were initially analyzed with Sequencher version 3.0 (Gene Codes Corp., Ann Arbor, MI) followed by further compilation and analysis using the Wisconsin Sequence Analysis Package version 9.1 (Genetics Computer Group, Inc., Madison, WI).

Phylogenetic Analysis

Phylogenetic analysis was performed with the PAUP version 3.1.1 and PAUP* version 4.0 Macintosh computer software programs [Swofford, 1998]. Maximum parsimony analysis was conducted on nucleotide sequence alignments by using a 3:1 weighting of transversions to transitions. Neighbor-joining analysis was carried out by using uncorrected and distance correction models (Kimura-2 parameter and Jukes-Cantor). Amino acid sequences were analyzed by using maximum parsimony with the step matrix PROTPARS, part of the PAUP version 3.1.1 and PAUP* version 4.0 software packages [Swofford, 1998].

RESULTS

Analysis of Diagnostic PCR Fragments

RT and nested PCR analysis using primers designed to detect *Sigmodontinae*-associated hantaviruses [Johnson et al., 1997] yielded products of the expected size for the S segment and M segment G1-encoding (M-G1) fragments for all three samples. However, only the sample from Mato Grosso state (CAS) produced a product from the G2 region of the M segment (M-G2). Primers designed to amplify the equivalent G2 region of BAY virus were used successfully with the two samples from São Paulo state. The primer sequences were removed from the PCR product sequences, and the remaining sequences (394, 259, and 139 nt in length for S, M-G1, and M-G2, respectively) were compared for each of the PCR fragments. Comparison of the 394-nt piece of S segment showed only a 4.8% nt and a 0.8% amino acid (aa) difference between ARA and FRA. However, when ARA and FRA virus sequences were compared with those from CAS virus, 21.1–22.3% nt and 3.1–3.8% aa differences were observed. A similar pattern was seen with a 139-nt piece of the G2 region of M segment, showing 5.0% nt difference and identical aa sequence between ARA and FRA viruses and 17.3–18.0% nt and 6.5% aa difference when these sequences were compared with CAS virus. The 259-nt G1 fragment demonstrated the highest overall variability, with 4.6% nt and 3.5% aa difference observed between ARA and FRA viruses, and 27.0–27.8% nt and 12.8–14.0% aa difference seen between these viruses and CAS virus. Based on the high degree of identity seen between the ARA and FRA viruses, only the ARA and CAS virus samples were selected for further PCR and sequence analysis.

Phylogenetic and Genetic Analysis of the Partial M Segment Sequence

A 1734-nt piece of M segment (mostly G2 encoding) sequence was generated for both ARA and CAS viruses and compared with the equivalent region of other hantaviruses (Fig. 2A). ARA and CAS viruses form a well-supported clade (98% bootstrap support) along with other South American hantaviruses on phylogenetic analysis of the nucleotide sequences using maximum parsimony with 3:1 weighting of transversions over transitions. More specifically, LN virus [Johnson et al., 1997] forms the most ancestral branch, with the Brazilian and previously characterized Argentinean hantaviruses (AND [Edelstein et al., unpublished]; Hu39694, LEC, and ORN viruses [Levis et al., 1998]) forming a well-supported clade (84%). Unfortunately, the exact topology among the Brazilian and Argentinean viruses is not clear from analysis based on the nucleotide sequence data, except for moderate support (70%) placing Hu39694, LEC, and ORN viruses together. Analysis using the neighbor-joining method, uncorrected and incorporating the distance models of Kimura-2 parameter and Jukes-Cantor, was conducted for comparison (data not shown). All three models estimated phylogenies that are consistent with that generated by maximum parsimony analysis, displaying high support for the Brazilian and Argentinean viruses forming a monophyletic clade, and showing LN virus as a more ancestral taxon. Again, further resolution was not possible within the Brazilian/Argentinean clade because of less than 65% bootstrap support for any of the branches. The two Brazilian viruses showed 21.5% nt difference to each other. The range of nucleotide difference between the Brazilian and Argentinean hantaviruses was 19.4–21.1% for CAS virus and 20.0–22.0% for ARA virus, while the Argentinean viruses displayed between 17.2% and 20.1% difference among themselves. Both the Brazilian and Argentinean viruses exhibited 22.2–23.5% nt difference to the more ancestral LN virus and 24.9–27% nt difference to the phylogenetically next closest neighbors, BAY and BCC viruses.

Maximum parsimony analysis using the amino acid sequence (578 aa) deduced from the M segment fragment sequences helped resolve some of the more weakly supported nodes seen using the nucleotide data (Fig. 2B). For instance, the monophyly of the South American hantaviruses and BCC and BAY viruses was supported in 76% of bootstrap replicates. Within the South American clade, LN virus again formed the ancestral node, followed by ARA, CAS, and the Argentinean viruses, all with more than 75% bootstrap support. Amino acid pairwise comparisons showed that the CAS and ARA viruses were quite distinct, displaying 8.0% difference. Amino acid differences of 5.0–7.5% were noted between the Brazilian and Argentinean viruses with 2.1–5.2% aa difference seen among the Argentinean group alone. Both groups of viruses demonstrated

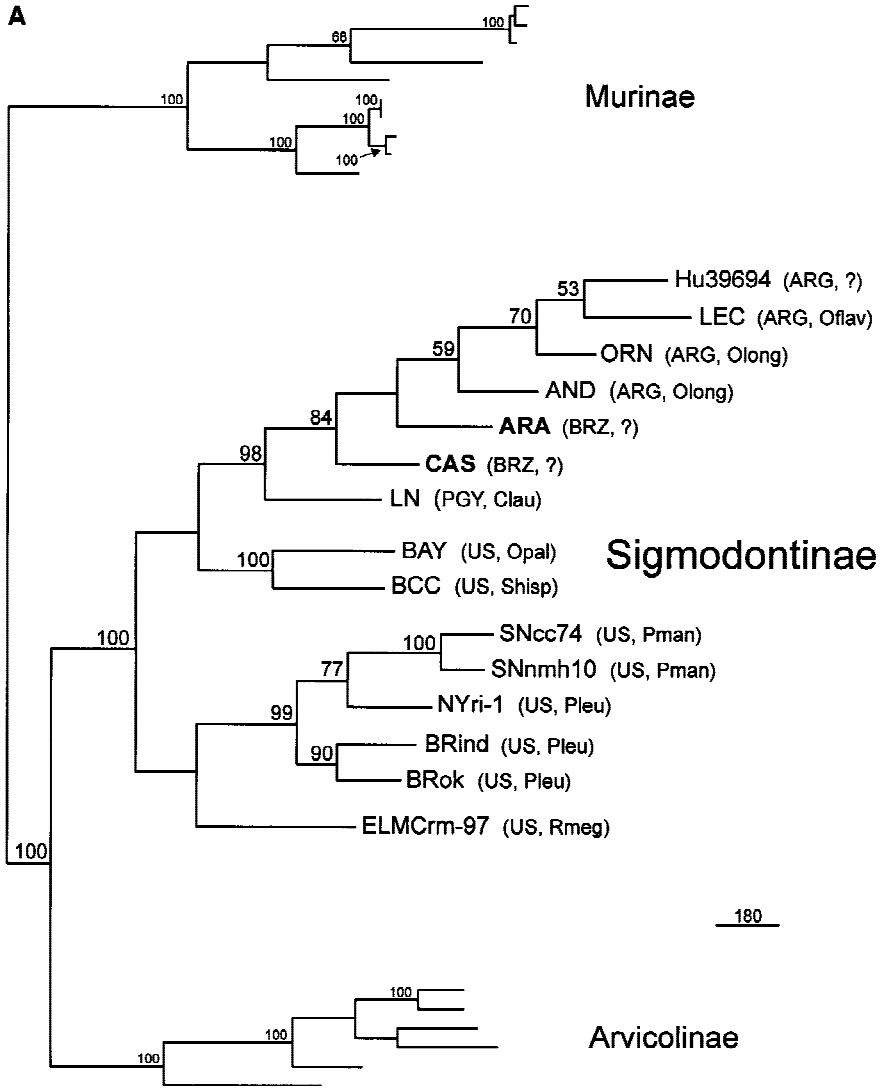

Fig. 2. **A:** Phylogenetic analysis using a 1734-nt region of the M segment (mostly G2 sequence) from the Brazilian hantaviruses Araraquara (ARA) and Castelo dos Sonhos (CAS), and the corresponding region from previously characterized hantaviruses. Maximum parsimony analysis with 3:1 weighting of transversions over transitions was performed with PAUP* 4.0. The heuristic search option was used and generated a single most parsimonious tree. Some 500 bootstrap replicates were conducted and values above 50% are shown at branch points. Horizontal distances represent nucleotide step differences (see bar scale), whereas vertical branches are for visual clarity only.

8.9–10.2% aa difference relative to LN virus and even higher differences (14.9–17.5%) to BAY and BCC viruses.

A 139-nt sequence fragment had been generated earlier from a virus [referred to as Juquitiba (JUQ)] detected in autopsy tissue from the original HPS cases discovered just south of São Paulo city, Brazil, in 1993 [Nichol et al., 1996; da Silva et al., 1997; Vasconcelos et al., 1997; Monroe et al. 1999]. This sequence region was compared with that now available for the new Brazilian CAS and ARA viruses and showed 20.9% nt/4.4% aa and 21.6% nt/2.2% aa difference, respectively. Comparison of this small G2 fragment with those of the previously characterized South American hantaviruses showed that JUQ virus was actually closer to the Argentinean hantaviruses LEC, ORN, and Hu39694, displaying 17.3%, 18.0%, and 18.7% nt difference, respectively, and no deduced amino acid differences.

Genetic Analysis of Virus S Segment Fragments

A 643-nt portion of S segment was obtained for the Brazilian CAS and ARA viruses and maximum parsimony analysis carried out using a 3:1 weighting of transversions over transitions. The estimated phylogeny was compatible with that obtained for the M segment data set, but lacked good resolution for many of the nodes (Fig. 3). Again, the Argentinean AND virus [López et al., 1997] was monophyletic with the Brazilian ARA and CAS viruses (72% bootstrap support), with ARA appearing ancestral to the other two. Furthermore, LN virus from Paraguay [Johnson et al., 1997] and RIOM virus from Bolivia [Bharadwaj et al.,

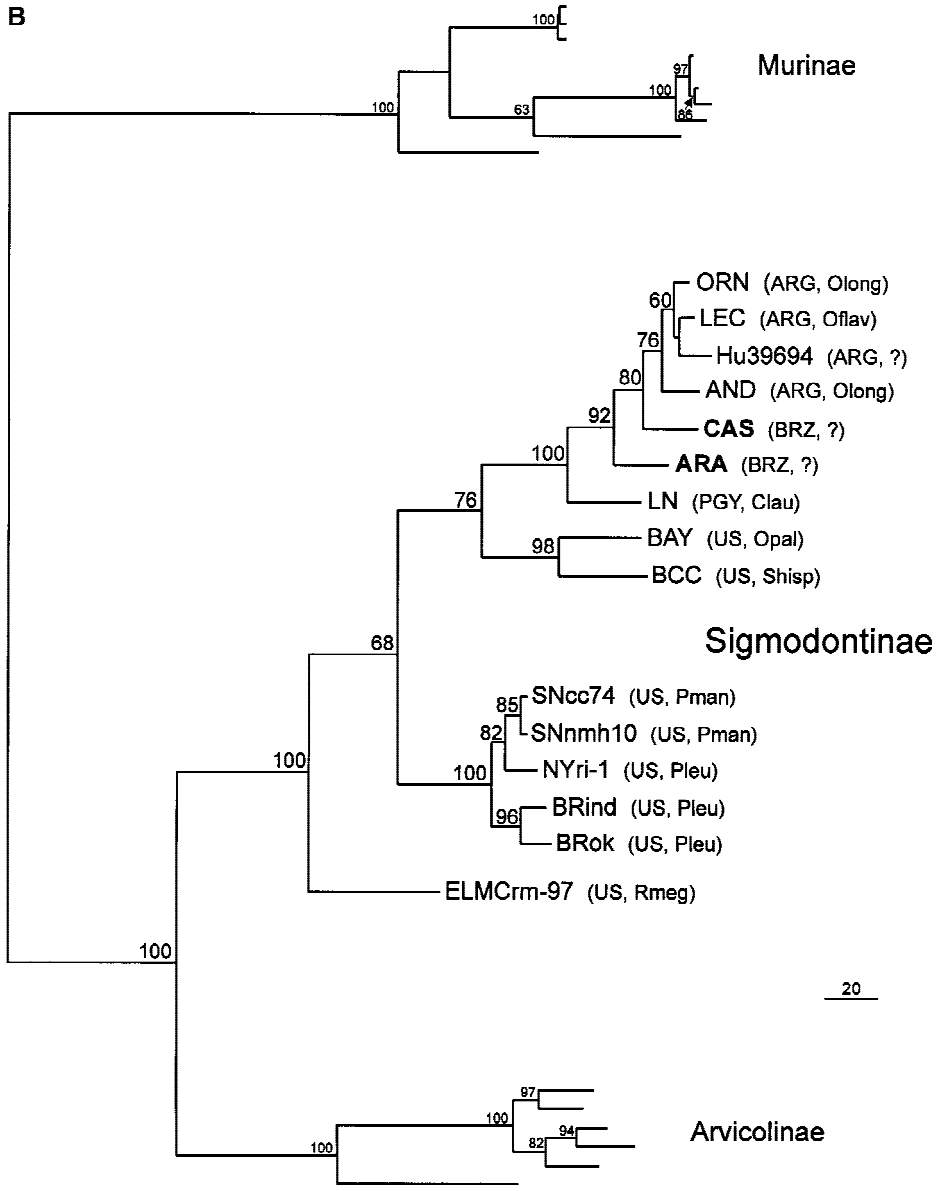

Fig. 2. Continued. **B:** Maximum parsimony analysis of the deduced 578-aa M segment piece obtained for the Brazilian viruses ARA and CAS and the corresponding region of other hantaviruses. The heuristic search option was used and two most parsimonious trees were found that differed only in the estimated topology of the *Murinae*-associated hantaviruses. Country of origin and rodent host (if known) for the sigmodontine viruses are indicated in parentheses. Abbreviations include Hu39694, Human 39694; LEC, Lechiguanas; ORN, Oran; AND, Andes; ARA, Araraquara; CAS, Castelo dos Sonhos; LN, Laguna Negra; BAY, Bayou; BCC, Black Creek Canal; SN, Sin Nombre; NY, New York; BR, Blue River; ELMC, El Moro Canyon; ARG, Argentina; BRZ, Brazil; PGY, Paraguay; US, United States; ?, unknown; Oflav, *Oligoryzomys flavescens*; Olong, *Oligoryzomys longicaudatus*; Clau, *Calomys laucha*; Opal, *Oryzomys palustris*; Shis, *Sigmodon hispidus*; Pman, *Peromyscus maniculatus*; Pleu, *Peromyscus leucopus*; Rmeg, *Reithrodontomys megalotis*. The partial M segment nucleotide and amino acid analyses included SEO B-1 [Isegawa et al., 1990], SR-11 [Arikawa et al., 1990], and 80-39 [Antic et al., 1991]; Thailand [Xiao et al., 1994]; DOB [Avsic-Zupanc et al., 1995]; HTN 76-118, HoJo and Lee [Schmaljohn et al., 1988], CUMC-B11 (unpublished, GenBank accession U37729), and HV114 [Xiao et al., 1993]; BAY [Morzunov et al., 1995]; BCC [Ravkov et al., 1995]; Hu39694, LEC and ORN [Levis et al., 1998]; AND [Edelstein et al., unpublished]; LN [Johnson et al., 1997]; Blue River Indiana and Oklahoma [Morzunov et al., 1998]; SN CC-74 [Schmaljohn et al., 1995] and NM H10 [Spiropoulou et al., 1994]; NY RI-1 [Hjelle et al., 1995d]; El Moro Canyon RM-97 [Torrez-Martinez et al., 1995]; PUU Vindeln/L20Cg/83 [Horling et al., 1995], Vranica [Reip et al., 1995], Sotkamo [Vapalahti et al., 1992], CG1820 [Giebel et al., 1989], and 90-13 [Bowen et al. 1995]; Prospect Hill [Parrington et al., 1991].

1997] were found to be sister taxa. A neighbor-joining (uncorrected distances) analysis of the same data resulted in a similar tree topology for among the South American hantaviruses, but with less support for the CAS/AND clade (62%) and more support for the LN/RIOM clade (99%) and the overall South American group (71%) (data not shown). Unlike the M segment analysis, maximum parsimony analysis using the amino acid sequence data did not help improve the resolution of the tree topology (data not shown). Direct comparison of the S segment sequences of CAS and ARA viruses showed them to exhibit 18.2% nt and 5.6%

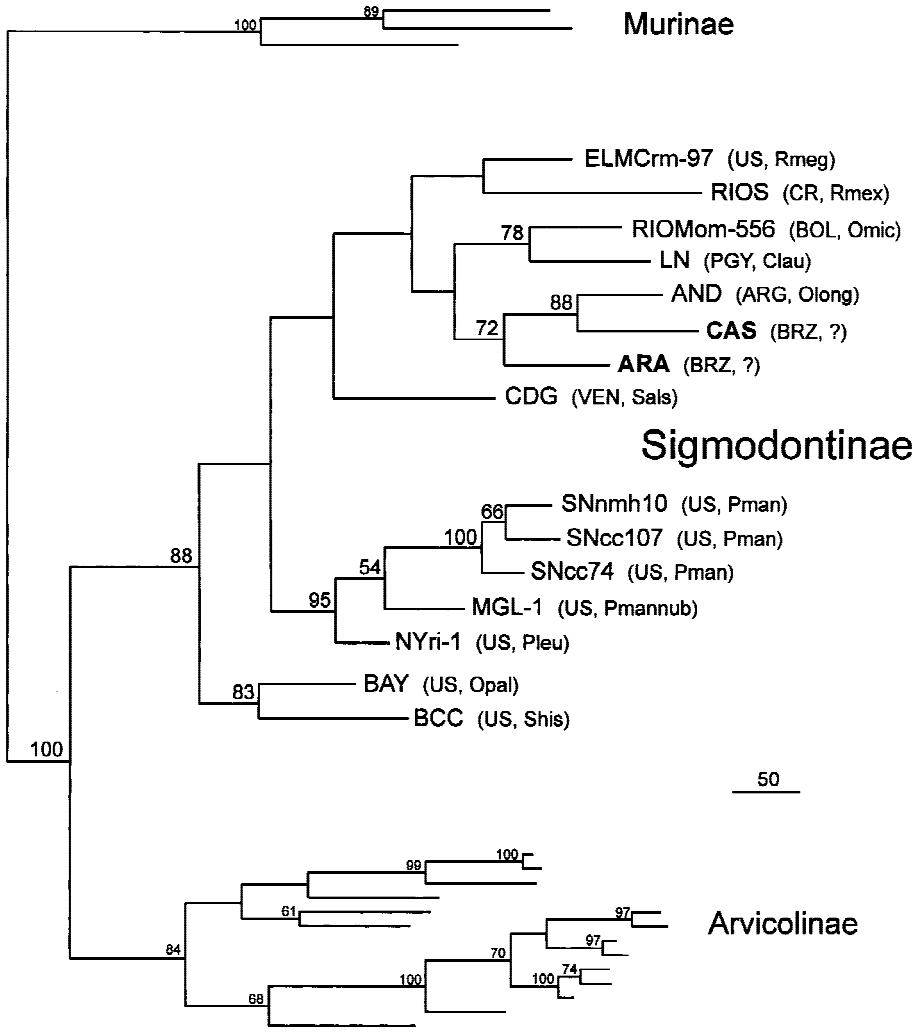

Fig. 3. Phylogenetic analysis of a 643-nt region of the S segment from the Brazilian hantaviruses Araraquara (ARA) and Castelo dos Sonhos (CAS) and the corresponding region from previously characterized hantaviruses. Analysis was conducted by using maximum parsimony with 3:1 weighting of transversions over transitions. Analysis was performed with PAUP* using the heuristic search option. Some 500 bootstrap replicates were carried out and values above 50% are shown at branch points. Two most parsimonious trees were generated with differences only in the topology of some of the *Arvicolinae*-associated hantaviruses. Horizontal distances represent nucleotide step differences (see bar scale), while vertical branches are for visual clarity only. Country of origin and rodent host (if known) for the sigmodontine viruses are indicated in parentheses. Abbreviations used: ELMC, El Moro Canyon; RIOS, Rio Segundo; RIOM, Rio Mamore; LN, Laguna Negra; AND, Andes; CAS, Castelo dos Sonhos; ARA, Araraquara; CDG, Caño Delgadito; SN, Sin Nombre; MGL, Monongahela; NY, New York; BAY, Bayou; BCC, Black Creek Canal; US, United States; CR, Costa Rica; BOL, Bolivia; PGY, Paraguay; ARG, Argentina; BRZ, Brazil; VEN, Venezuela; Rmeg, *Reithrodontomys megalotis*; Rmex, *Reithrodontomys mexicanus*; Omic, *Oligoryzomys microtis*; Clau, *Calomys laucha*; Olong, *Oligoryzomys longicaudatus*; ?, unknown; Sals, *Sigmodon alstoni*; Pman, *Peromyscus maniculatus*; Pmannub, *Peromyscus maniculatus nubiterrae*; Pleu, *Peromyscus leucopus*; Opal, *Oryzomys palustris*; Shis, *Sigmodon hispidus*. The partial S segment nucleotide analysis included HTN 76–118 [Schmaljohn et al., 1986]; DOB [Avsic-Zupanc et al., 1995]; SEO SR-11 [Arikawa et al., 1990]; El Moro Canyon RM-97 [Hjelle et al., 1994]; Rio Segundo [Hjelle et al., 1995a]; Rio Mamore (RIOM) OM-556 [Bharabwaj et al., 1997]; LN [Johnson et al., 1997]; AND [López et al., 1997]; Caño Delgadito [Fulhorst et al., 1997]; SN NM H10 [Spiropoulou et al., 1994], CC107, and CC74 [Schmaljohn et al., 1995]; Monongahela-1 [Song et al., 1996]; NY RI-1 [Hjelle et al., 1995c]; BAY [Morzunov et al., 1995]; BCC [Ravkov et al., 1995]; Tula Malacky/Ma370/94 [Sibold et al., 1995], Moravia/5293Ma/94 and 76Ma/87 [Plyusnin et al., 1995a]; Isla Vista PC-SB-77 [Song et al., 1995]; Prospect Hill [Parrington and Kang, 1990]; prairie vole hantavirus MO46 (GenBank accession U19303); PUU Vindeln/L20Cg/83 [Horling et al., 1995], Vranica [Reip et al., 1995], CG1820 [Stohwasser et al., 1990], Udmurtia/894Cg/91 and Evo/12Cg/93 [Plyusnin et al., 1995b], Sotkamo [Vapalahti et al., 1992], Virrat/25Cg/95 [Plyusnin et al., 1997], and 90-13 [Bowen et al., 1995]; Khabarovsk [Horling et al., 1996].

aa differences. The Brazilian virus S segment sequences were both quite close to that of AND virus, with only 14.5–15.1% nt and 1.9–3.7% aa difference evident. The LN and RIOM viruses display 13.8% nt and 2.3% aa differences between themselves, and 17.3–19.6% nt and 6.5–9.3% aa differences relative to ARA, CAS, and AND viruses.

DISCUSSION

Serological surveys based on immunofluorescent assays carried out in the 1980s indicated that hantaviruses were present in South America [Weissenbacher et al., 1990, 1996; Vasconcelos et al., 1992; Iversson et al., 1994], and Seoul virus was isolated from a rat from

Belem, Brazil, in 1982 [LeDuc et al., 1985]. However, it was not until the discovery of SN virus and HPS in North America in early 1993 [Nichol et al., 1993] that more intensive surveillance efforts led to the detection of HPS cases and associated hantaviruses in South America. By early 1998, it had become clear that HPS was a greater problem in South America than in North America, with the confirmation of more than 230 HPS cases in Argentina, Brazil, Chile, Paraguay, and Uruguay [Pan American Health Organization, 1998].

The data presented here show that at least three genetically distinguishable virus lineages (JUQ, ARA, and CAS) are associated with HPS cases in Brazil. As has been seen elsewhere with other hantaviruses (e.g., Plyusnin et al. [1996]), the data presented here suggest that there is some correlation between virus genetic diversity and geographic distance between case locations within Brazil. Rodent trapping and identification efforts are currently under way to attempt to discover the rodent hosts of JUQ, ARA, and CAS viruses. It appears likely that these viruses will be maintained by different sigmodontine rodent species or subspecies, as the nucleotide and deduced amino acid sequence differences seen between the JUQ, ARA, and CAS virus lineages are compatible with those seen among previously characterized distinct hantaviruses associated with different rodent host species or subspecies [Plyusnin et al., 1996; Schmaljohn and Hjelle, 1997; Johnson et al., 1997]. Interestingly, on pairwise sequence comparisons, the JUQ, ARA, and CAS virus lineages are each closer to Argentinean viruses than they are to each other, suggesting each may be hosted by rodents similar to their closest virus relatives in Argentina.

Virus phylogenetic analysis confirms the strong overall correlation between the hantavirus and rodent host genetic relationships, which have been suggested previously to indicate that hantaviruses have likely coevolved with rodent hosts over several million years [Plyusnin et al., 1996; Morzunov et al., 1998; Nichol, 1999]. Interestingly, the phylogenetic tree based on glycoprotein amino acid sequence data places the BAY and BCC viruses from *Oryzomys palustris* in Louisiana and *Sigmodon hispidus* in Florida, respectively, firmly in the clade containing all the viruses of South American origin (with 78% bootstrap support), and not with the other viruses of North American origin. However, this finding is consistent with the coevolution concept, as these rodent species are considered to be part of the South American sigmodontine complex, which is genetically distinct from the North American neotomine–peromycine complex [Engel et al., 1998]. One exception to the coevolutionary pattern can be seen from the S segment phylogenetic tree, where RIOM virus (from *Oligoryomys microtis* in Bolivia) is found to form a well-supported clade (78% bootstrap support) with LN virus (from *Calomys laucha* in Paraguay) and not with Andes virus, the representative of other *Oligoryomys*-borne viruses. As has been suggested earlier for NY and Topografov viruses [Morzunov et al., 1998; Vapalahti et al., 1999], such data are suggestive of a host-switching event.

Consistent with the earlier genetic studies [López et al., 1996, 1997; Fulhorst et al., 1997; Johnson et al., 1997; Levis et al., 1998], the phylogenetic studies presented here illustrate the difficulty of accurately estimating the evolutionary relationships of the various South American HPS-associated and sigmodontine-hosted hantaviruses, despite analyzing large fragments of the virus genome. A similar situation arises when trying to estimate the molecular phylogeny of the rodents themselves. These patterns are consistent with the hypothesized rapid adaptive radiation and speciation of subfamily *Sigmodontinae* rodents in South America following the introduction of their ancestors by waif dispersal over water from Central America approximately 5–9 million years ago [Engel et al., 1998]. The complexity of the South American hantavirus picture is likely to increase over the next few years, given the speciose nature of the subfamily *Sigmodontinae*. The predominantly South American sigmondontines alone, include 61 genera and 299 species [Engel et al., 1998], making it inevitable that additional hantaviruses will be identified in this region, several of which are likely to be associated with HPS.

ACKNOWLEDGMENTS

We thank Giselda Katz and Ali Khan for background information on the cases, Akemi Suzuki for important fieldwork assistance and discussion, and Mike Bowen for helpful comments.

REFERENCES

Antic D, Lim BU, Kang CY. 1991. Molecular characterization of the M genome segment of the Seoul 80-39 virus; nucleotide and amino acid sequence comparisons with other hantaviruses reveal the evolutionary pathway. Virus Res 19:47–58.

Arikawa J, Lapenotiere HF, Iacono-Connors L, Wang MG, Schmaljohn CS. 1990. Coding properties of the S and the M genome segments of Sapporo rat virus: comparison to other causative agents of hemorrhagic fever with renal syndrome. Virology 176:114–125.

Avsic-Zupanc T, Toney A, Anderson K, Chu YK, Schmaljohn CS. 1995. Genetic and antigenic properties of Dobrava virus: a unique member of the *Hantavirus* genus, family *Bunyaviridae*. J Gen Virol 76:2801–2808.

Bharadwaj M, Botten J, Torrez-Martinez N, Hjelle B. 1997. Rio Mamore virus: genetic characterization of a newly recognized hantavirus of the pygmy rice rat, *Oligoryzomys microtis*, from Bolivia. Am J Trop Med Hyg 57:368–374.

Bowen MD, Kariwa H, Rollin PE, Peters CJ, Nichol ST. 1995. Genetic characterization of a human isolate of Puumala hantavirus from France. Virus Res 38:279–289.

da Silva MV, Vasconcelos MJ, Hidalgo NTR, Veiga APR, Canzian M, Marotto PC, de Lima VC. 1997. Hantavirus pulmonary syndrome. Report of the first three cases in São Paulo, Brazil. Revista do Instituto de Medicina Tropical de São Paulo 39:231–234.

Elliott RM, Schmaljohn CS, Collett MS. 1991. Bunyaviridae genome structure and gene expression. Curr Top Microbiol Immunol 169: 91–141.

Engel SR, Hogan KM, Taylor JF, Davis SK. 1998. Molecular systematics and paleobiology of the South American sigmodontine rodents. Mol Biol Evol 15:35–49.

Fulhorst CF, Monroe MC, Salas RA, Duno G, Utrera A, Ksiazek TG, Nichol ST, de Manzione NMC, Tovar D, Tesh RB. 1997. Isolation, characterization, and geographic distribution of Caño Delgadito virus, a newly discovered South American hantavirus (family Bunyaviridae). Virus Res 51:159–171.

Giebel LB, Stohwasser R, Zoeller L, Bautz EKF, Darai G. 1989. Determination of the coding capacity of the M genome segment of nephropathia epidemica virus strain Hällnäs B1 by molecular cloning and nucleotide sequence analysis. Virology 172:498–505.

Hjelle B, Anderson B, Torrez-Martinez N, Song W, Gannon WL, Yates TL. 1995a. Prevalence and geographic genetic variation of hantaviruses of New World harvest mice (*Reithrodontomys*): identification of a divergent genotype from a Costa Rican *Reithrodontomys mexicanus*. Virology 207:452–459.

Hjelle B, Chavez-Giles F, Torrez-Martinez N, Yates T, Sarisky J, Webb J, Ascher M. 1994. Genetic identification of a novel hantavirus of the harvest mouse *Reithrodontomys megalotis*. J Virol 68:6751–6754.

Hjelle B, Jenison SA, Goade DE, Green WB, Feddersen RM, Scott AA. 1995d. Hantaviruses: clinical, microbiologic, and epidemiologic aspects. Crit Rev Clin Lab Sci 32:469–508.

Hjelle B, Krolikowski J, Torrez-Martinez N, Chavez-Giles F, Vanner C, Laposata E. 1995b. Phylogenetically distinct hantavirus implicated in a case of hantavirus pulmonary syndrome in the northeastern United States. J Med Virol 46:21–27.

Hjelle B, Lee SW, Song W, Torrez-Martinez N, Song JW, Yanagihara R, Gavrilovskaya I, Mackow ER. 1995c. Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse, *Peromyscus leucopus*: genetic characterization of the M genome of New York virus. J Virol 69:8137–8141.

Horling J, Cheng Y, Plyusnin A, Persson K, Lehvaslaiho H, Vaheri A, Niklasson B, Lundkvist Å. 1995. Nucleotide and deduced amino acid sequences of the M and S genome segments of a Swedish Puumala virus isolate. Virus Res 39:321–330.

Horling J, Chizhikov V, Lundkvist A, Jonsson M, Ivanov L, Dekonenko A, Niklasson B, Dzagurova T, Peters CJ, Tkachenko E, Nichol S. 1996. Khabarovsk virus: a phylogenetically and serologically distinct *Hantavirus* isolated from *Microtis fortis* trapped in far-east Russia. J Gen Virol **77**:687–694.

Isegawa Y, Fujiwara Y, Ohshima A, Fukunaga R, Murakami H, Yamanishi K, Sokawa Y. 1990. Nucleotide sequence of the M genome segment of hemorrhagic fever with renal syndrome virus strain B-1. Nucleic Acids Res 18:4936.

Iversson LB, Travassos da Rosa APA, Rosa MDB, Lomar AV, Sasaki M da GM, LeDuc JW. 1994. Human infection by Hantavirus in southern and southeastern Brazil. Revista da Associacao Medica Brasileira 40:85–92.

Johnson AM, Bowen MD, Ksiazek TG, Williams RJ, Bryan RT, Mills JN, Peters CJ, Nichol ST. 1997. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. Virology 238:115–127.

LeDuc JW, Smith GA, Pinheiro FP, Vasconcelos PFC, Rosa EST, Maiztegui JI. 1985. Isolation of a Hantaan-related virus from Brazilian rats and serologic evidence of its widespread distribution in South America. Am J Trop Med Hyg 34:810–815.

Levis S, Morzunov SP, Rowe JE, Enria D, Pini N, Calderon G, Sabattini M, St. Jeor SC. 1998. Genetic diversity and epidemiology of hantaviruses in Argentina. Journal of Infectious Disease 177:529–538.

López N, Padula P, Rossi C, Lazaro ME, Franze-Fernandez MT. 1996. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology 220:223–226.

López N, Padula P, Rossi C, Miguel S, Edelstein A, Ramírez E, Franze-Fernández MT. 1997. Genetic characterization and phylogeny of Andes virus and variants from Argentina and Chile. Virus Res 50:77–84.

Monroe MC, Morzunov SP, Johnson AM, Bowen MD, Artsob H, Yates T, Peters CJ, Rollin PE, Ksiazek TG, Nichol ST. 1999. Genetic diversity and distribution of *Peromyscus*-borne hantaviruses in North America and comparison with other hantaviruses. Emerg Infect Dis 5:75–86.

Morzunov SP, Feldmann H, Spiropoulou CF, Semenova VA, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST. 1995. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. J Virol 69:1980–1983.

Morzunov SP, Rowe JE, Ksiazek TG, Peters CJ, St. Jeor SC, Nichol ST. 1998. Genetic analysis of the diversity and origin of hantaviruses in *Peromyscus leucopus* mice in North America. J Virol 72: 57–64.

Nichol ST. 1999. Genetic analysis of hantaviruses and their host relationships. In: Saluzzo JF, Dodet B, editors. Emergence and control of rodent-borne viral diseases. Paris: Elsevier. In press.

Nichol ST, Ksiazek TG, Rollin PE, Peters CJ. 1996. Hantavirus pulmonary syndrome and newly described hantaviruses in the United States. In: Elliott RM, editor. The Bunyaviridae. New York: Plenum. p 269–280.

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ. 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262:914–917.

Pan American Health Organization. 1998. Hantaviruses in the Americas: guidelines for diagnosis, treatment, prevention, and control. Washington, DC: Pan American Health Organization.

Parrington MA, Kang CY. 1990. Nucleotide sequence analysis of the S genomic segment of Prospect Hill virus: comparison with the prototype hantavirus. Virology 175:167–175.

Parrington MA, Lee PW, Kang CY. 1991. Molecular characterization of the Prospect Hill virus M RNA segment: a comparison with the M RNA segments of other hantaviruses. J Gen Virol 72:1845–1854.

Plyusnin A, Cheng Y, Vapalahti O, Pejcoch M, Unar J, Jelinkova Z, Lehväslaiho H, Lundkvist Å, Vaheri A. 1995a. Genetic variation in Tula hantaviruses: sequence analysis of the S and M segments of strains from Central Europe. Virus Res 39:237–250.

Plyusnin A, Horling J, Cheng Y, Kanerva M, Mustonen J, Vapalahti O, Niemimaa J, Henttonen H, Niklasson B, Lundkvist Å, Vaheri A. 1997. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J Clin Microbiol 35:1090–1096.

Plyusnin A, Vapalahti O, Lehväslaiho H, Apekina N, Mikhailova T, Gavilovskaya I, Laakkonen J, Niemimaa J, Henttonen H, Brummer-Korvenkontio M, Vaheri A. 1995b. Genetic variation of wild Puumala viruses within the serotype, local rodent populations and individual animals. Virus Res 38:25–41.

Plyusnin A, Vapalahti O, Vaheri A. 1996. Hantaviruses: genome structure, expression, and evolution. J Gen Virol 77:2677–2687.

Ravkov EV, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST. 1995. Genetic and serologic analysis of Black Creek Canal virus and its association with human disease and *Sigmodon hispidus* infection. Virology 210:482–489.

Reip A, Haring B, Sibold C, Stohwasser R, Bautz EK, Darai G, Meisel H, Kruger DH. 1995. Coding strategy of the S and M genomic segments of a hantavirus representing a new subtype of the Puumala serotype. Arch Virol 140:2011–2026.

Schmaljohn AL, Li D, Negley DL, Bressler DS, Turell MJ, Korch GW, Ascher MS, Schmaljohn CS. 1995. Isolation and initial characterization of a newfound hantavirus from California. Virology 206: 963–972.

Schmaljohn C, Hjelle B. 1997. Hantaviruses: a global disease problem. Emerg Infect Dis 3:95–104.

Schmaljohn CS, Arikawa J, Hasty SE, Rasmussen L, Lee HW, Lee PW, Dalrymple JM. 1988. Conservation of antigenic properties and sequences encoding the envelope proteins of prototype Hantaan virus and two virus isolates from Korean haemorrhagic fever patients. J Gen Virol 69:1949–1955.

Schmaljohn CS, Jennings GB, Hay J, Dalrymple JM. 1986. Coding strategy of the S genome segment of Hantaan virus. Virology 155: 633–643.

Sibold C, Sparr S, Schulz A, Labuda M, Kozuch O, Lysy J, Krueger DH, Meisel H. 1995. Genetic characterization of a new hantavirus detected in *Microtis arvalis* from Slovakia. Virus Genes 10:277–281.

Song JW, Baek LJ, Nagle JW, Schlitter D, Yanagihara R. 1996. Genetic and phylogenetic analyses of hantaviral sequences amplified from archival tissues of deer mice (*Peromyscus maniculatus nubiterrae*) captured in the eastern United States. Arch Virol 141:959–967.

Song W, Torrez-Martinez N, Irwin W, Harrison FJ, Davis R, Ascher M, Jay M, Hjelle B. 1995. Isla Vista virus: a genetically novel hantavirus of the California vole *Microtis californicus*. J Gen Virol 76:3195–3199.

Spiropoulou CF, Morzunov S, Feldmann H, Sanchez A, Peters CJ, Nichol ST. 1994. Genome structure and variability of a virus causing hantavirus pulmonary syndrome. Virology 200:715–723.

Stohwasser R, Giebel LB, Zoeller L, Bautz EKF, Darai G. 1990. Molecular characterization of the RNA S segment of Nephropathia epidemica virus strain Haellnaes B1. Virology 174:79–86.

Swofford DL. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0. Sunderland, MA: Sinauer.

Torrez-Martinez N, Song W, Hjelle B. 1995. Nucleotide sequence analysis of the M genomic segment of El Moro Canyon hantavirus: antigenic distinction from Four Corners hantavirus. Virology 211: 336–338.

Vapalahti O, Kallio-Kokko H, Salonen EV, Brummer-Korvenkontio M, Vaheri A. 1992. Cloning and sequencing of Puumala virus Sotkamo strain S and M RNA segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. J Gen Virol 73:829–838.

Vapalahti O, Lundkvist Å, Fedorov V, Conroy C, Hirvonen S, Plyusnina A, Nemirov K, Fredga K, Cook J, Niemimaa J, Kaikusalo A, Henttonen H, Vaheri A, Plyusnin A. 1999. Isolation and characterization of a hantavirus from *Lemmus sibiricus*: evidence for host-switch during hantavirus evolution. J Virol 73:5586–5592.

Vasconcelos MI, Lima VP, Iversson LB, Rosa MDB, Travassos da Rosa APA, Travassos da Rosa ES, Pereira LE, Nassar E, Katz G, Matida LH, Zaparoli MA, Ferreira JJB, Peters CJ. 1997. Hantavirus pulmonary syndrome in the rural area of Juquitiba, São Paulo metropolitan area, Brazil. Revista do Instituto de Medicina Tropical de São Paulo 39:237–238.

Vasconcelos PFC, Travassos da Rosa ES, Travassos da Rosa APA, Travassos da Rosa JFS. 1992. Evidence of circulating hantaviruses in Brazilian Amazonia through high prevalence of antibodies in residents of Manaus, Brazil. Ciencia e Cultura 44:162–163.

Weissenbacher MC, Cura E, Segura EL, Hortal M, Baek LJ, Chu YK, Lee HW. 1996. Serological evidence of human hantavirus infection in Argentina, Bolivia, and Uruguay. Medicina (Buenos Aires) 56: 17–22.

Weissenbacher MC, Merani MS, Hodara VL, de Villafane G, Gajdusek DC, Chu YK, Lee HW. 1990. Hantavirus infection in laboratory and wild rodents in Argentina. Medicina (Buenos Aires) 50:43–46.

Xiao SY, LeDuc JW, Chu YK, Schmaljohn CS. 1994. Phylogenetic analyses of virus isolates in the genus *Hantavirus*, family *Bunyaviridae*. Virology 198:205–217.

Xiao SY, Liang M, Schmaljohn CS. 1993. Molecular and antigenic characterization of HV114, a hantavirus isolated from a patient with haemorrhagic fever with renal syndrome in China. J Gen Virol 74:1657–1659.